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Dispersing *Bacillus thuringiensis* for Control of Cankerworm in Shelterbelts[1]

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A hydraulic sprayer and a cold fogger were equally effective for dispersal of the entomogenous bacterium *Bacillus thuringiensis* Berliner in shelterbelts. Pyrocide (pyrethrum) and Dimilin (a growth regulator) in combination with or without the bacterium provided the greatest cankerworm mortality in the belts.

Keywords: *Bacillus thuringiensis,* insect control.

The spring cankerworm, *Paleacrita vernata* (Peck), and the fall cankerworm, *Alsophila pometaria* (Harr), are important defoliators in shelterbelts in the northern Great Plains. In recent years, *P. vernata* has been the dominant species. Because they seldom kill trees, cankerworms are not considered a major forest pest. However, severely defoliated shelterbelts do not protect adjacent crops from severe weather stresses, and are esthetically unpleasing as well.

Concern over the use of conventional insecticides dictates that other types of control, including biological agents, be investigated. In a recent review, Harper (1974) indicated that 4 International Units[3] per acre of the entomogenous bacterium *Bacillus thuringiensis* Berliner effectively reduced cankerworm populations. Falcon et al. (1974) concluded that vertical coverage of cotton plants was more effective when *B. thuringiensis* was applied from the ground with a cold fogger than when the bacterium was applied from the air.

In 1975 we evaluated the effectiveness of ground-based fogging equipment for dispersing the bacterium in shelterbelts, and the bacterium in combination with other nonconventional insecticides. The study, conducted near Walhalla in Pembina County, North Dakota, was a cooperative effort involving North Dakota State University and the USDA Forest Service, Rocky Mountain Forest and Range Experiment Station.

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[3]*An International Unit is defined as 1000 times the amount of a standard* B. thuringiensis *preparation required to kill 50 percent of the test larvae (usually the cabbage looper,* Trichoplusia ni*) in a laboratory assay, divided by the amount of the test material required to kill 50 percent of a group of similar larvae (Burges 1967).*

Methods and Materials[4]

The study was conducted in six Siberian elm (*Ulmus pumila* L.) shelterbelts. The belts, approximately 0.805 km long and 10.7 m tall, were utilized as blocks. Nine, 10-tree plots, 15.2 m apart, were marked in each belt. Treatments were randomized in each belt.

Larvae were counted on twigs approximately 0.5 m long before and after spraying. Twigs were cut from four sampling sites in each of two trees in each plot on May 28 and June 9, 1975. Two sites were located approximately 6.1 meters from ground level, one on each side of the crown; two sites were located at eye level, one on each side of the crown. Larval density per 100 Siberian elm leaves was determined for each plot and was based on the equation $Y = 763.1 X^2$ where Y is the number of leaves per one-half meter twig, and X is the twig diameter in centimeters. Eighty-nine percent of the variation in the number of leaves was due to twig diameter. Mortality of cankerworm larvae was based on larvae per 100 leaves. Counts from the two trees were averaged by site for analytical purposes.

A test utilizing a known number of cankerworms was conducted to compare the effectiveness of the equipment described below for applying *B. thuringiensis* to shelterbelts. Sleeve cages (0.3048 m by 0.762 m) constructed from 22 mesh (22 openings/2.54 centimeters) Lumite screen (fig. 1) were placed over 1 branch in each plot (a total of 24 cages) to which *B. thuringiensis* was applied with different types of equipment and in untreated check plots immediately following application. Twenty-five third-instar larvae were placed on the foliage in each cage.

Nonconventional insecticides used in the investigation included Thuricide 16B (*B. thuringiensis*), Pyrocide (pyrethrum), and Dimilin (the growth regulator TH 6040). The materials were applied June 3,

[4]*The use of trade names is for brevity and specificity, and does not imply endorsement by the U.S. Department of Agriculture or North Dakota State University to the exclusion of other equivalent products.*

Figure 1.—Sleeve cage (0.3048 by 0.762 cm) used to isolate 25 third instar cankerworm larvae on Siberian elm foliage in a control test.

1975, at the following rates per acre: Thuricide 16B at 0.946 liter of product (4.0 billion I.U.), Pyrocide at 0.946 liter of product, and Dimilin 2E at 0.113 kilogram actual insecticide. When insecticides were combined in a treatment they were used at the same rates. The insecticides were applied with a cold fogger (Microgen M52W-15), a thermal fogger (Dyna Fog Model 70B) (fig. 2) and a hydraulic sprayer (Spartan). Each type of equipment was calibrated to determine the time required to apply specific amounts of insecticide to a known volume of foliage. The crown volume was determined by a method described by Stein and Doran (1975). Insecticides were applied from a pickup truck driven at a constant speed of 4.83 km/hr. Equivalents of approximately 18.87 and 2.83 liters of spray per acre were applied with the hydraulic sprayer and foggers, respectively.

Dispersal and survival of *B. thuringiensis* spores were determined from five 0.3167 cm^2 leaf samples by means of a serial dilution technique. Leaf samples were collected on June 4, 1975, 24 hours after application of the bacterium. The samples were placed in 10 ml of sterile, distilled water and shaken in a vortex mixer for 15 seconds. Serial dilutions were prepared in sterile, distilled water and spread-plated in replicate on five plates of plate count agar (Difco). Plates were incubated at room temperature. Average counts of viable spores from the replicates were recorded. Samples were collected from three sites (approximately 1.83, 3.66, and 6.1 m above ground) from the spray side of a single tree in each plot treated with *B. thuringiensis*. The samples were stored in coolers prior to processing. Results were subjected to analyses of variance, range tests (Duncan 1955), and regression analysis.

Figure 2.—Cold fogger (rear) and thermal fogger (front) used to apply *Bacillus thuringiensis*.

Results and Discussion

Differences in cankerworm populations among belts and sampling sites in trees prior to treatment were highly significant (table 1). Early instar larval density was greater in the top half of the trees (sites 1 and 2).

There were no significant differences in cankerworm numbers among treatment plots in the belts, and interaction between plots and sample sites was negligible.

Mortality of cankerworms exposed to *B. thuringiensis* and other insecticides varied with the types of equipment and combinations of insecticides used (table 2).

In the top half, spray side (site 1), pyrethrum plus *B. thuringiensis* and Dimilin plus *B. thuringiensis* applied with the hydraulic sprayer killed the most cankerworms (87 percent in both cases). In the top half, lee side of the tree (site 2), pyrethrum and pyrethrum plus *B. thuringiensis* applied with the hydraulic sprayer were the most effective (97 and 87 percent mortality, respectively). Pyrethrum plus *B. thuringiensis* applied with the cold fogger were the most effective treatments at site 3 in the lower half, spray side of the tree (96 and 91 percent mortality, respectively). In the lower half, lee side of the tree (site 4) Dimilin plus pyrethrum applied with the hydraulic sprayer and *B. thuringiensis* applied with the cold fogger were the most effective (96 and 94 percent mortality, respectively). Overall pyrethum plus *B. thuringiensis,* Dimilin plus pyrethrum, *B. thuringiensis,* pyrethrum and Dimilin plus *B. thuringiensis* applied by the hydraulic sprayer were the most effective. *Bacillus thuringiensis* applied with the hydraulic sprayer and the foggers was also effective.

Reasons for improved cankerworm control with the combination treatments are not clear at this time. Possible explanations include increased efficacy due to additive effects of components, and/or protection of the bacterium by the other formulations. A comparison indicated that overall mortality in belt 1 was lower than in other belts.

Mortality of cankerworms on treated foliage under sleeve cages indicates that the hydraulic sprayer and cold fogger were equally effective for dispersing *B. thuringiensis* spores. The thermal fogger was not as effective (table 3).

Spore coverages for different types of application equipment, and for combinations of *B. thuringiensis* with pyrethrum and Dimilin are shown in table 4.

Coverage appears best when combinations of *B. thuringiensis* with pyrethrum and Dimilin were applied by hydraulic sprayer. Spore coverage was also good when *B. thuringiensis* alone was applied by cold fogger or hydraulic sprayer. Statistically, the first four treatments were the same. The thermal fogger gave the lightest coverage.

The relationship of cankerworm mortality to *B. thuringiensis* spore density on foliage was not as strong as expected. Only 9.5 percent of the variation of cankerworm mortality was due to variation in spore counts. Considerable rain fell within 24 hours after the bacterium was applied and periodically throughout the sampling period (June 4-July 9,

Table 1.--Mean number of cankerworm larvae per 0.5 m twig before application of insecticide in Siberian elm shelterbelts, Walhalla, North Dakota, 1975

Belts		Sites[1] in sample trees	
Number	Larvae[2]	Number	Larvae[2]
5	44.96 a	1	28.19 a
3	36.92 ab	3	26.92 ab
6	31.96 bc	4	22.32 b
2	27.42 c	2	21.94 b
4	19.68 d		
1	5.26 e		

[1]Sites 1 and 2 were approximately 6.1 meters from the ground, one on each side of the crown (spray and lee sides); sites 3 and 4 were at eye level, one on each side of the crown.

[2]Means followed by the same letter do not differ significantly at P = 0.05.

Table 2.--Mortality of cankerworms on Siberian elm foliage treated with *Bacillus thuringiensis* applied with several types of equipment and in combination with other insecticides, Walhalla, North Dakota, 1975

Treatment: Material(s)	Treatment: Dispersal method	Mean mortality[1]	Mean mortality[2]
		- - *percent* - -	
Dimilin + pyrethrum	Hydraulic sprayer	94 a	88
B. thuringiensis + pyrethrum	Hydraulic sprayer	93 a	87
Pyrethrum	Hydraulic sprayer	85 a	71
B. thuringiensis	Hydraulic sprayer	83 ab	67
B. thuringiensis + Dimilin	Hydraulic sprayer	80 ab	62
B. thuringiensis	Thermal fogger	78 ab	58
B. thuringiensis	Cold fogger	75 ab	53
Dimilin	Hydraulic sprayer	74 bc	50
Untreated		47 c	--

[1]Means followed by the same letter do not differ significantly at P = 0.05.
[2]Adjusted by Abbott's formula (Abbott 1925), for the untreated check.

Table 3.--Mortality of 25 caged cankerworm larvae placed on Siberian elm foliage after *Bacillus thuringiensis* treatment with several types of equipment, Walhalla, North Dakota, 1975

Application method	Mean mortality[1]	Adjusted mortality[2]
	- - - - - *percent*	- - - - -
Hydraulic sprayer	81.3 a	74
Cold fogger	80.0 a	72
Thermal fogger	66.7 b	54
Untreated check	27.3 c	--

[1]Means followed by the same letter do not differ significantly at P = 0.05.
[2]Adjusted by Abbott's formula (Abbott 1925) to determine mortality attributable for the untreated check.

Table 4.--Coverage of Siberian elm foliage with *Bacillus thuringiensis* spores applied with several types of equipment and in combination with other insecticides, Walhalla, North Dakota, 1975

Treatment		Mean spore count[1,2]
Material(s)	Dispersal method	
B. thuringiensis + pyrethrum	Hydraulic sprayer	1,731,667 a
B. thuringiensis + Dimilin	Hydraulic sprayer	1,582,222 a
B. thuringiensis	Hydraulic sprayer	1,170,689 a
B. thuringiensis	Cold fogger	1,160,589 ab
B. thuringiensis	Thermal fogger	556,033 b

[1]Based on five 0.3167 cm^2 samples of elm foliage from six replications per treatment.
[2]Means followed by the same letter do not differ significantly at $P = 0.05$.

1975). Windspeed was 4.83 to 8.05 km/hr. This weather is typical of early summer and control methods will have to adapt to it.

Buettner (1951) showed that the amount of ultraviolet radiation reaching the earth decreases as sky cover (clouds and haze) increases. Ultraviolet radiation inactivates bacterial spores. It is likely that weather conditions influenced treatment performance and spore populations on the foliage. Weather records from Walhalla are missing for the test period.

Survival of *B. thuringiensis* spores on Siberian elm foliage dropped rapidly during the first 48 hours (June 4-6) of exposure to weather (fig. 3). Inactivation continued into early July, followed by a stabilization of the decline in the number of viable spores. This is evidence that more susceptible spores were killed during the first 48 hours, and the spores remaining on the foliage after that period were more resistant to weather factors, especially ultraviolet radiation. Davis et al. (1968) described a logarithmic survival curve of microorganisms irradiated by ultraviolet light that tails off into a line whose slope corresponds to more resistant organisms. More sensitive organisms are inactivated first, and at the end only the most resistant survive. There were significant differences in spore survival associated with the treatments.

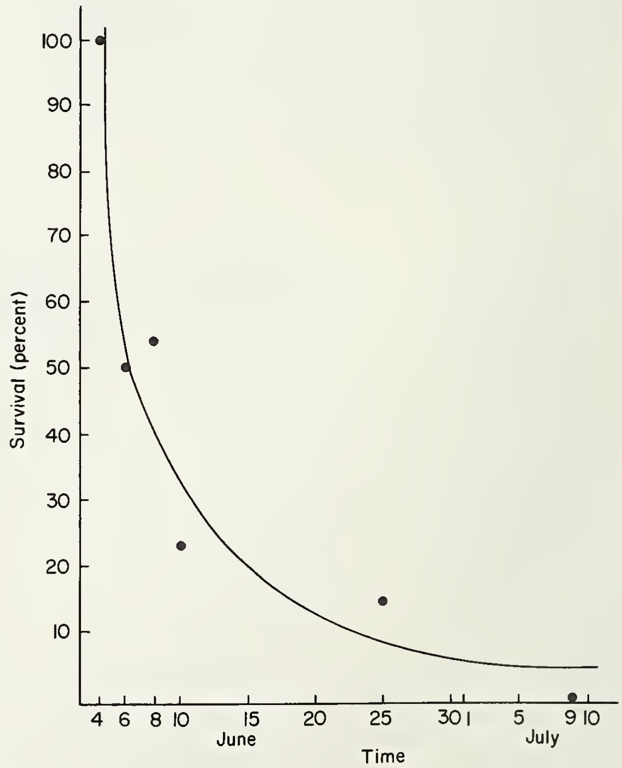

Figure 3.—Survival of *Bacillus thuringiensis* spores on Siberian elm foliage.

Summary and Conclusions

We evaluated the effectiveness of ground-based fogging equipment for dispersing the entomogenous bacterium *Bacillus thuringiensis* Berliner (in Thuricide 16B) for cankerworm control in Siberian elm shelterbelts. The bacterium was also tested in combination with other nonconventional insecticides. Both the spring cankerworm and the fall cankerworm were present in the plots near Walhalla, North Dakota.

Pyrocide (pyrethrum) plus Dimilin (a growth regulator) and *B. thuringiensis* plus pyrethrum applied with a hydraulic sprayer reduced cankerworm populations the most. Using a hydraulic sprayer to apply *B. thuringiensis* plus pyrethrum, or *B. thuringiensis* plus Dimilin, gave the best coverage of foliage with *B. thuringiensis* spores. The hydraulic sprayer and cold fogger both provided good dispersal of the bacterium when applied alone, and effectively controlled cankerworms.

When logistics are considered (amount of water needed, ease of handling), the cold fogger would be a good choice for applying the bacterium for cankerworm control in shelterbelts in the northern Great Plains.

There were no significant differences between treatments in survival of *B. thuringiensis* spores. Variability in the tests was rather high, except under controlled conditions where equal numbers of larvae (25) were used with all treatments.

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Although this report discusses research involving pesticides, such research does not imply that the pesticide has been registered or recommended for the use studied. Registration is necessary before any pesticide can be recommended. If not handled or applied properly, pesticides can be injurious to humans, domestic animals, desirable plants, fish, and wildlife. Always **read** and **follow** the directions on the pesticide container.

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